NOTES

RAPID METHODS FOR THE DETECTION OF MOTILITY

ROBERT A. GREENE, ELTON F. BLUM, CAMILLO T. DECORO, RAYNE B. FAIRCHILD, MILTON T. KAPLAN, JACK L. LANDAU, AND THOMAS R. SHARP

Department of Bacteriology, College of Osteopathic Physicians and Surgeons, Los Angeles, California

Received for publication May 21, 1951

When small tubes (75 by 10 mm), containing 1 or 2 ml of SIM medium (Difco) or Trypticase Agar Base (semisolid) (BBL), are preheated to 37 C, "stab-inoculated" from a young agar slant culture, and incubated in a water bath at 37 C, motility may be observed in a fraction of the time required by the methods usually employed with these media. In a series of cultures which were studied, motility could be determined usually within 90 to 120 minutes.

When small tubes (75 by 10 mm), containing 1 ml of nutrient broth, are preheated to 37 C and heavily inoculated from young nutrient agar slants or from colonies growing on agar plates, motility can be observed in hanging drop preparations within 15 to 30 minutes. In some cases, the freshly inoculated tubes may show motility.

FURTHER STUDIES ON THE SIGNIFICANCE OF SPIROCHETAL GRANULES

EDWARD G. HAMPP[1]

National Institute of Dental Research, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Maryland

Received for publication May 28, 1951

The significance of the spirochetal granule in the life history of the spirochete has not been elucidated. It has been suggested that these granules may be germinative units by Balfour (Brit. Med. J., **1,** 752, 1911), Noguchi (J. Exptl. Med., **14,** 99, 1911), Leishman (Ann. inst. Pasteur, **32,** 49, 1918), Mudd *et al.* (J. Bact., **46,** 15, 1943), Hampp (J. Am. Dent. Assoc., **33,** 201, 1946), and Hampp *et al.* (J. Bact., **56,** 755, 1948). Other investigators are undecided or hesitant in accepting this hypothesis. Topley and Wilson (William Wood and Company, Baltimore, 1936) have indicated that these granules are artifacts and probably small particles of culture medium adhering to the spirochetes. Hampp (1946) and Hampp *et al.* (1948) have shown both by dark-field examination and by electron microscopy of pure cultures of certain of the oral spirochetes

[1] Fellow, Research Council, American Dental Association.

and cultures of alleged strains of *Treponema pallidum* that these spirochetal granules are a definite phase in the development of spirochetes and they are not artifacts. Additional support of the hypothesis has been contributed by Hampp (1946) by the fact that pure cultures of the smaller oral treponemes maintained in anaerobic jars at 37 C up to 31 months and exhibiting only refractile spirochetal granules by dark-field examination have given rise to normal growth in a limited period of time when transferred to fresh medium. It is the purpose of this paper to present further information on the survival of the smaller oral treponemes on aging of pure cultures of these organisms.

The spirochetes employed in this study were all small oral treponemes and designated as strains N36, N39, and N40. These organisms were removed from primary plates and subcultured for the first time by stab method in a medium described elsewhere (Hampp: J. Am. Dent. Assoc., **30,** 1066, 1943). The basic medium was enriched at the time of use by the addition of 10 per cent ascitic fluid and 0.1 per cent glutathione. First transfers were purposely employed for this experiment instead of old stock strains in order to rule out the possibility of the organisms becoming adapted to the medium over a long period of time thus increasing the chances for survival under the conditions of the experiment. The cultures were incubated under anaerobic conditions at 37 C and after 3 days were examined for growth. Certain of the cultures were purposely sampled with a capillary pipette with a bore of 2 mm in diameter and a 5 cm long column of agar removed as for transferring procedures. This was done in order to determine whether or not cultures of organisms that had been disturbed would survive the period selected for testing viability of the spirochetes. The cultures were returned to the anaerobic jars and incubated at 37 C. The jars were not opened again during the experimental period.

Cultures of the three strains of oral spirochetes were removed from the anaerobic jars after 74 months. Strain N39 was in pairs; one culture had been sampled at the beginning of the experiment, the other had not been molested. Strain N36 was unsampled, whereas, strain N40 had been sampled. In those cases in which an agar plug had been removed, the medium had split open through the center of the spirochetal growth from the top to the bottom of the column of agar contained in the test tubes. The cultures were sampled and wet preparation observed by dark-field illumination; as far as could be determined the cultures consisted of nothing but spirochetal granules and no vegetative forms of the organisms were in evidence. Fresh subcultures were prepared in the same type medium employed in the experimental cultures, and the stab inoculations were made with capillary pipettes of the pasteur type. The subcultures were placed in anaerobic jars and incubated at 37 C. The spirochetal cultures were examined after 48 hours and thereafter at 24-hour periods until growth became apparent. The unsampled strains of organisms, strains N39 and N36, became grossly positive at 72 hours and 84 hours, respectively. These observations were confirmed by dark-field examination of wet preparation of organisms. The sampled mate of N39 and the single strain N40 showed no evidence of growth even after 14 days of incubation; fresh subcultures were made and observed without evi-

dence of growth. This process was repeated on four separate occasions without success. Apparently the manipulation of cultures prior to storage had a detrimental effect on the perpetuation of the spirochetal strains.

The survival of the smaller oral treponemes, strains N39 and N36, for 74 months under these conditions adds credence to the assumption that the spirochetal granules may be germinative units in the life cycle of the spirochetes. It is a possibility that these granules may be resting bodies formed in response to adverse environmental conditions with reduction of their metabolic activities to a minimum with retention of reproductive capacities. Additional studies are in progress to determine how long spirochetal cultures can survive under the conditions described in these experiments.

THE USE OF A SYNTHETIC RESIN IN ANAEROBIC MEDIA

B. MALIN AND R. K. FINN

Departments of Bacteriology and Chemistry, University of Illinois, Urbana, Illinois

Received for publication June 13, 1951

Relatively few convenient media are available for the cultivation of anaerobes and some of these are unsuitable for particular applications. For example, it has been observed that the commonly used medium incorporating thioglycolate is inhibitory to some organisms in the presence of carbohydrate. The commercial availability of an oxygen adsorbing synthetic resin, "duolite S-10"[1] (Mills and Dickinson: Ind. Eng. Chem., **41,** 2842, 1949), suggested the possible use of such a resin in media for culturing anaerobes. This insoluble resin can be autoclaved satisfactorily and is noninhibitory to the organisms tested.

For testing anaerobic media a variety of *Clostridium* species were used: *C. thermosaccharolyticum*, *C. acetobutylicum*, *C. chauvei*, *C. perfringens*, *C. sporogenes*, *C. pasteurianum*, and *C. putrificum*. The basal medium consisted of peptone, phosphate, and a carbohydrate. To this were added one or more of the following in various combinations: 0.1 per cent agar, 0.1 per cent sodium thioglycolate, and the resin (about 1 g wet weight per 10 ml).

Growth experiments were successful using basal medium containing both agar and the resin, and basal medium plus resin alone. In the latter medium, a flocculent growth was obtained directly above the resin layer. It was possible to separate the growth from the resin by shaking the test tube, thus dispersing the cells throughout the medium. The cells could then be removed easily by decantation or with a pipette since the resin settled almost immediately.

Media containing thioglycolate gave poor or no growth with *C. thermosaccharolyticum*, *C. chauwei*, *C. putrificum*, and *C. perfringens*.

Although "duolite S-10" was the only resin used in these experiments, it seems reasonable that other anion exchange resins of amine type would perform equally

[1] Purchased from the Chemical Process Company, Redwood, California.